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Growth Responses of Potted *Vitis vinifera* Cultivars Differ to a Mycorrhizal Inoculant and Phosphorus Fertilizer

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Abstract. **Biological amendments, such as arbuscular mycorrhizal (AM) fungal inoculant products, are increasingly incorporated into agricultural management plans as a way to improve plant productivity. However, the effects of mycorrhizal inoculants on plant growth are context-dependent and can vary with soil fertility and among plant cultivars. To optimize the use of mycorrhizal inoculant products on wine grapes at the nursery stage, we tested the effect of a mycorrhizal inoculant product with and without the addition of phosphorus (P) fertilizer on the growth and tissue nutrients of two popular *Vitis vinifera* cultivars, Merlot and Chardonnay. We rooted dormant cuttings in the following respective treatments: no AM fungal inocula or P fertilizer; AM fungal inocula; P fertilizer; and co-amendment of AM fungal inocula and P fertilizer. We grew the grapevines in pots for 5 months in a greenhouse. Growth responses to treatments differed between cultivars. 'Merlot' vines had a stronger growth response to the mycorrhizal inoculant product than 'Chardonnay', especially when no P fertilizer was added. The co-amendment of AM fungi and P fertilizer resulted in larger root biomass for 'Merlot', but there was no effect of any treatment on the root biomass of 'Chardonnay'. 'Merlot' vines grown with the AM fungal inoculant product also had higher tissue P than uninoculated vines, but there was no effect of inoculation on tissue nutrients of 'Chardonnay'. This study provides evidence of grapevine cultivar-specific responses to an AM fungal inoculant product in a greenhouse, which may be useful when planning nursery management strategies for the incorporation of biological amendments into grapevine production.**

Grape (*Vitis* sp.) is a globally distributed woody perennial crop with a long history of cultivation (Iland et al. 2011). Depending on the final product use (juice, table wine, raisin) and management, a planted vine could have a commercial production lifespan of 20 to 100 or more years. This production lifespan is important because the typical time for the return on investment for a US vineyard can range from 5 to more than 10 years, depending on the regional and intended vine use (Awondo et al. 2017; Hyde 2010; Olen and Skinkis 2018; Washington State Wine Association 2023). The dominant influencing factors for those enterprise budgets are first-year vineyard establishment costs and the time until the vineyard is able to produce a sellable crop. Most vineyards typically require 4 years or more before they can produce a fully realized crop. Therefore, during the establishment of new vineyards, steps that can either reduce those initial costs or speed the time to vine establishment can reduce the time to achieve a return on investment.

Although there has been increasing interest from growers in using biological products to improve grapevine growth and nutrient status, biological inoculants such as AM fungi are known to have varied effects on plant productivity, and the results of AM fungal inoculation may not be consistent across growing conditions or among plant cultivars (Bennett and Groten 2022; Hoeksema et al. 2010). The AM fungi form symbiotic relationships with plant roots, and in a bidirectional exchange, plants provide carbon (C) to mycorrhizal fungi, and fungal hyphae increase plant access to nitrogen (N), P, and water (Smith and Read 2008). However, the availability of nutrients in soil can significantly impact the outcome of the mutualistic exchange (Johnson et al. 1997). For instance, AM fungi often benefit plant growth in soils with low available P (Smith and Read 2008), but these benefits can be reduced under conditions with high soil available P (Schreiner 2007). Grapevines are strongly mycotrophic (Possingham and Groot Obbink 2017), and inoculations with AM fungi have been shown to increase shoot length and P uptake in potted Pinot noir (*Vitis vinifera*) vines in a greenhouse study (Schreiner 2007). However, plant–mycorrhizal interactions are dynamic, and the extent to which growth responses to an AM fungal inoculant product vary among wine grape cultivars is not well-understood, especially at the early stages of plant development and under nursery production conditions.

The objective of this study was to determine how different cultivars of wine grapes respond to a mycorrhizal inoculant product with and without the addition of P fertilizer. Therefore, we performed a greenhouse experiment using dormant, newly rooted cuttings of two popular wine grape (*Vitis vinifera*) cultivars, Merlot and Chardonnay, that received no AM fungal or P fertilizer amendment, AM fungal inoculant product, P fertilizer, or a co-amendment with both the AM fungal inoculant product and P fertilizer. Vines were grown in a greenhouse for 5 months and data of vine growth (shoot length), above and belowground biomass, percentage AM fungal colonization of roots, and tissue nutrients were collected. We predicted that the AM fungal inoculant product (MycoBloom; MycoBloom LLC, Lawrence, KS, USA) would increase vine growth and tissue nutrients in both cultivars, and that the benefits of inoculation would be highest in the vines that received no P fertilizer. We questioned whether 'Merlot' and 'Chardonnay' vines may vary in their responses to inoculation because cultivar-specific responses to AM fungal inoculation have been observed in other crops, such as *Zea mays* (corn) (Khalil et al. 1994), and there is a general lack of cultivar-specific AM fungal information for *V. vinifera*. Understanding how mycorrhizal inoculant products impact the growth and tissue nutrients of different cultivars in a greenhouse setting will be useful for informing management strategies aimed at optimizing the use of biological amendments for wine grape production at the nursery stage.

Materials and Methods

Plant material. We obtained callused, unrooted cuttings of 'Chardonnay' (*V. vinifera;* FPS selection 79.1) and 'Merlot' (*V. vinifera*; FPS selection 15) from Inland Desert Nursery (Benton City, WA, USA). We chose these cultivars because they are widely grown and popular in local vineyards; in addition, most of the *V. vinifera* acreage in Washington is planted to own-rooted, nongrafted vines. The callused, unrooted cuttings were rooted in situ to their experimental pots; this ensures roots were not precolonized with AM fungi before the experiment. Plants were grown in 4-L pots on greenhouse benches [Washington State University (WSU), Richland, WA, USA] in a randomized complete block design for

5 months. The experiment was established on 2 Oct 2019, and the vines were destructively removed from pots on 2 Mar 2020. There were a total of 15 vine replicates of each treatment (2 cultivars × 4 treatments × 15 replicates = 120 experimental units). Each pot contained a single plant.

Soil. We collected field soil (Warden silt loam) from a local agricultural field (46.2544118, −119.7283880) near existing research vineyards at the WSU Irrigated Agricultural Research and Extension Center (Prosser, WA, USA). Properties and nutrients for the field soil included the following: pH 7.9; 4 ppm nitrate; 1.5 ppm ammonium; 31 ppm Olsen P; and 214 ppm potassium (Soiltest Farm Consultants, Inc., Moses Lake, WA, USA) (Supplemental Table S1). We mixed the field soil 1:1 (by volume) with medium-course landscaping sand (Beaver Bark, Richland, WA, USA) to improve drainage and autoclaved it twice (121 °C for 2 h, rest for 24 h) to eliminate resident soil organisms, including pests and pathogens. Properties and nutrients for the autoclaved sand:soil mix included the following: pH 8.0; 2.6 ppm nitrate; 7.1 ppm ammonium; 10 ppm Olsen P; and 271 ppm potassium (Soiltest Farm Consultants, Inc.) (Supplemental Table S1). All pots in the experiment contained the same autoclaved sand:soil substrate to which the AM fungal inoculant and/or P fertilizer was added, which is a common method for assessing the plant growth response to mycorrhizal fungal inoculants (Cheeke et al. 2019; Reynolds et al. 2006).

Arbuscular mycorrhizal fungal inoculant product. We used MycoBloom as the mycorrhizal inoculant product for the AM fungal inoculation treatment (MycoBloom LLC), which included the following AM fungal species: *Acaulospora spinosa*; *Cetraspora pellucida*; *Claroideoglomus claroideum*; *Claroideoglomus lamellosum*; *Entrophospora infrequens*; *Funneliformis mosseae*; and *Racocetra fulgida*. MycoBloom was used as the inocula for this experiment for the following reasons: it is widely available for purchase from online retailers; it contains seven different AM fungal species that have been shown to improve the growth of a variety of perennial plant species (Bauer et al. 2017; Cheeke et al. 2019), including those in perennial agroecosystems (Koziol et al. 2019); the fungi in the inocula were isolated from perennial plants and thus may associate well with perennial grapevines; some of the fungal species in the inoculant are also found in vineyards, including *E. infrequens*, *C. claroideum*, and *F. mosseae* (Cheng and Baumgartner 2004); and the AM fungi in this inoculant product have been used successfully to promote plant growth in more than 15 peer-reviewed scientific publications (Cheeke et al. 2019; Koziol and Bever 2016), demonstrating the viability of the product. This is important because some commercial mycorrhizal inoculant products fail to establish, even when added to sterilized soil/substrate, thus yielding no experimental treatment or effects (Salomon et al. 2022). Each inoculated pot in our experiment had 400 cm^3 of the fungal inoculant (10% inoculation rate by volume) added to the rooting zone of each vine, and the remainder of the pot contained autoclaved sand:soil mix (Supplemental Fig. S1). Although this is higher than the 2.5% inoculation rate suggested for container gardening on the MycoBloom website, we used a 10% inoculation rate for this study for the following reasons: it is within the range of effective inoculation rates reported for greenhouse studies that tested the perennial plant growth response to AM fungal inoculation (Cheeke et al. 2019); other studies have shown that higher application rates of inoculant products are often needed to be effective (Koziol et al. 2019); and not all spores/propagules in formulated products may be viable at the time of application. By adding the same amount of inocula to the rooting zone of each vine, we were able to reduce the variation in the amount of AM fungi that each pot received because heterogeneity and patchiness can occur when trying to mix large batches of soil with inocula before assembling the experimental units. Moreover, because the inocula was added at the rooting zone at planting and covered with autoclaved sand:soil mix, the chance of the fungal treatment contaminating other pots in the greenhouse was reduced (e.g., via splashing, dust, or cross-contamination while measuring). The symbiotic relationship between plant roots and AM fungi is dynamic, and the fungal hyphae will continue to grow and colonize roots over the course of the experiment through the production of fungal hyphae. The uninoculated control pots contained only autoclaved sand:soil mix. We did not add autoclaved MycoBloom to the control pots because autoclaving is known to release a flush of nutrients from killed organisms and through chemical changes to substrates under high temperature and pressure (Anderson and Magdoff 2005; Berns et al. 2008; Skipper and Westermann 1973). Although calcined clay was included as a filler in MycoBloom (calcined clay is sometimes added to inoculant products to maintain moisture in greenhouse pots), our plants were watered daily; therefore, the small amount of calcined clay in the inoculated pots was unlikely to affect plant growth over the course of the experiment. To account for potential effects of nonmycorrhizal microbes present in MycoBloom, each pot received 50 mL of a microbial filtrate prepared from the fungal inocula, which was filtered through a sieve (38 μm) and then through filter paper (5–10 μm), allowing bacteria to pass through, but not fungal spores, roots, or larger organisms.

Potted plant management. The daily temperature in the greenhouse was recorded digitally by Hobo data loggers (Onset, Cape Cod, MA, USA), and temperatures and humidity levels on each bench were monitored daily with benchtop digital recorders (AcuRite, Lake Geneva, WI, USA). The average greenhouse daily low temperature was 16 °C, and the average daily high was 28 °C. Humidity ranged from 20% to 40% during the growing period. Vines received additional lighting beginning at 76 d after planting to achieve a total photoperiod of 16 h from 1000-W high-pressure sodium bulbs. Vines were watered daily for 3 to 4 min using an automated drip irrigation system (drip rate: 25 mL/min). We managed powdery mildew (foliar and fruit fungal disease caused by *Erysiphe necator*) using a mineral oil spray per the manufacturer's guidelines for grapes (12 to 28 mL/L; 1.2%–2.8%; PureSpray GREEN, Intelligro, Canada) every 10 d, starting at the first sign of infection (∼80 d after planting). The dilute mineral oil spray was added directly to the surface of the leaves, and there was no direct contact between the foliar mineral oil spray and mycorrhizal fungi in the roots.

When most vines had three to four true leaves (∼20 d after planting), we added a P fertilizer used by local growers (NUE 0–30–0; 8.6 g P_2O_5/pot; BioGro, Mabton, WA, USA) to the P fertilizer treatments. Each vine was also fertilized with a P-free (15–0–15) fertilizer (0.08 g/pot; Simple Lawn Solutions, Lake Panasoffkee, FL, USA) 111 d after planting, which added N and K to the soil to reduce the potential for macronutrient deficiencies during the experiment. To reduce the potential for micronutrient deficiencies, we applied a foliar micronutrient treatment (∼1% each B, Cu, Fe, Mg, Mn, Zn; 0.26 mL/vine; BioGro, Mabton, WA, USA) twice thereafter using a hand-pumped sprayer.

Plant growth measurements. We recorded the initial bud number for each cutting at the time of planting to account for variations in size before treatment. This number was used as a covariate in the statistical analysis. We began collecting shoot length data 60 d after planting using a flexible measuring tape, and we measured from the base of the shoot to the tip of the apical meristem. We continued to record shoot length every 30 d for the remainder of the experiment. At 60 d, vines were pruned to one primary shoot that was trained onto a 1.2-m-high bamboo stake (Schreiner 2007).

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When the primary shoot reached the top of the stake, it was cut off to encourage lateral shoot growth instead of vertical shoot growth, and the following length measurements included lengths from lateral shoots (Schreiner 2007).

At 5 months after planting, we destructively harvested the vines and separated the roots from the shoots for biomass. The roots were thoroughly washed to remove all traces of soil and sand from the root system and blotted dry with a paper towel before recording fresh weights of the whole root system. Subsamples of fine roots were collected from multiple parts of the root system to ensure a representative subsample from each vine was collected to assess the percentage of mycorrhizal colonization of the roots. The fresh weights of the whole root system and the root subsample were recorded separately, and a dry weight conversion was used to add back the weight of the subsampled roots to obtain the total root biomass (g, dry weight) before the analysis. Shoots and roots were dried for 48 h at 70 °C to collect aboveground and belowground biomass data (g, dry weight). Plant tissue (leaf, petiole, green stem) was collected from all vines to determine nutrients, including N, P, K, and Ca (KUO Testing Laboratories, Pasco, WA, USA).

Mycorrhizal colonization. Roots were cleared and stained to observe fungal structures (modified from Phillips and Hayman 1970). Briefly, roots were cleared using 10% KOH (simmered for 20 min), soaked at room temperature in 5% bleach solution for 2 min to lighten the roots, acidified at room temperature in 5% lactic acid, and then stained at room temperature with 0.05% trypan blue in lactoglycerol. Root subsamples from each plant were cut into ~1-cm fragments and mounted onto microscope slides (one slide per plant; 120 slides in total). Roots were assessed for the presence or absence of AM fungal structures, including the hyphae, arbuscules, and vesicles, out of 100 intersections per sample using a compound microscope at 200× total magnification with a vertical crosshair in the eye piece for scoring each intersection (McGonigle et al. 1990). The percentage of colonization of AM fungi was determined as the number of intersections containing AM fungal structures out of 100 total root intersections analyzed.

Statistical analysis. Data were analyzed using R (R Core Team 2021). To calculate the mycorrhizal growth response of each cultivar, we used the following formula: $[(Biomass_{Inoculated} - Biomass_{Uninoculated})/Biomass_{Uninoculated}]*100$ for each P fertilizer treatment. The mycorrhizal growth response represents the relative change in aboveground biomass caused by the addition of AM fungal inocula in the different P fertilizer treatments. This resulted in a total of the following four mycorrhizal growth response values: 'Merlot' to AM fungi with no P fertilizer added; 'Merlot' to AM fungi with P fertilizer added; 'Chardonnay' to AM fungi with no P fertilizer added; and 'Chardonnay' to AM fungi with P fertilizer added. For example, a mycorrhizal growth response of 23 represents a 23% increase in the aboveground biomass of the inoculated vines compared with the uninoculated vines in a particular P fertilizer treatment.

We used linear mixed effects models in the "lme4" package (Bates et al. 2015) to test the effects of cultivar, mycorrhizal inocula, P fertilizer, and their interactions on vine shoot length, shoot biomass (leaves, petioles, stem), root biomass, root-to-shoot ratio, percentage of mycorrhizal colonization of the roots, and nutrients of vine tissue. Because 'Merlot' and 'Chardonnay' varied in their growth rates and their responses to the experimental treatments (Supplemental Table S2), and because we could not meet the assumption of equal variance within each group to include both cultivars in the same model, the final statistical analyses were conducted for each cultivar separately. To determine the effect of AM fungal inocula on vine growth in each P fertilizer treatment, we focused primarily on the AM fungal × P fertilizer interaction term in the model and the results of the a priori contrasts comparing growth response of the inoculated vines and uninoculated vines within each P fertilizer treatment.

In each linear mixed effects model, the initial bud number was included as a covariate to account for potential variations in the initial size of the vine cuttings before treatment, and the greenhouse block was included as a random effect to account for potential environmental variations. Because uninoculated vines contained no fungal structures in roots, only the inoculated vines were included in the models testing the effects of P fertilizer on AM fungal colonization for each cultivar. We tested the significance of terms in our linear mixed effects models using an analysisi of variance with the R package car (Fox and Weisberg 2011). Assumptions for each model were checked using a visual inspection of residuals from quantile–quantile plots. The effects of AM fungal inocula on vine growth and/or tissue nutrients were then decomposed into a priori contrasts that separately tested the average growth response of each cultivar to AM fungal inocula in each P fertilizer treatment using the estimated marginal means 'emmeans' package of R. Tukey adjustments were performed to limit the type 1 error rate caused by α-inflation during the contrast comparisons.

Results

Mycorrhizal growth response differed by cultivar

The 'Merlot' grapevines had a stronger growth response to the MycoBloom inoculant product than the 'Chardonnay' grapevines (Fig. 1), but the direction of the effect differed for 'Merlot' in each P fertilizer treatment. 'Merlot' vines were 23% larger when inoculated with MycoBloom than uninoculated 'Merlot' when grown in soil with no P fertilizer added ($P = 0.043$) (Supplemental Table S3, Fig. 1). When fertilized with P, however, inoculated 'Merlot' vines were 10% smaller compared with the uninoculated vines (Fig. 1), but not statistically smaller ($P = 0.341$) (Supplemental Table S3). 'Chardonnay' vines inoculated with MycoBloom grew 13% larger when not fertilized with P, and 2% larger when fertilized with P, compared with the uninoculated 'Chardonnay' vines (Fig. 1); however, these differences were not statistically significant ($P = 0.261$ and $P = 0.966$, respectively) (Supplemental Table S4). Therefore, despite the small increases in 'Chardonnay' biomass with AM fungal inoculation, the

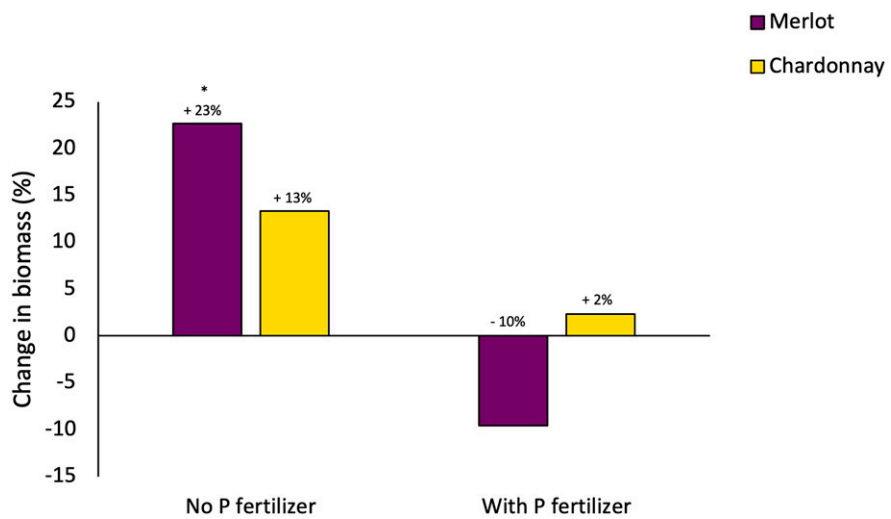

Fig. 1. Variations in mycorrhizal growth responses (% change in biomass) of young potted *Vitis vinifera* 'Merlot' (purple) and 'Chardonnay' vines (gold) to an arbuscular mycorrhizal (AM) fungal inoculant product (MycoBloom; MycoBloom LLC) without (left) or with (right) the addition of phosphorous (P) fertilizer. Plants were grown in 4-L pots in a greenhouse for 5 months. The mycorrhizal growth response represents the relative change in aboveground biomass caused by the addition of AM fungal inocula in each P fertilizer treatment. *$P = 0.043$. Estimated marginal (EM) means contrasts showing differences in shoot biomass between inoculated and uninoculated 'Merlot' vines in each P fertilizer treatment are included in Supplemental Table S3. EM means contrasts showing differences in shoot biomass between inoculated and uninoculated 'Chardonnay' vines in each P fertilizer treatment are included in Supplemental Table S4.

mycorrhizal growth response for this cultivar in both P fertilizer treatments was neutral.

Treatment effects on vine growth

Shoot length. Interactions of AM fungi × P fertilizer were detected for 'Merlot' shoot length (cm) at every timepoint measured over the course of the experiment, but the direction and magnitude of the effects differed over time (Fig. 2A, Supplemental Table S5). When vines were harvested 150 d after planting, inoculated 'Merlot' vines grown without P fertilizer had longer shoot lengths than uninoculated vines ($P = 0.022$) (Supplemental Table S3, Fig. 2A, circles), but there was no difference in the shoot length of 'Merlot' vines grown with P fertilizer ($P = 0.162$) (Supplemental Table S3, Fig. 2A, triangles). Interactions of AM fungi × P fertilizer were only detected for 'Chardonnay' at one time point (60 d) ($P = 0.018$) (Supplemental Table S6, Fig. 2B); the inoculated 'Chardonnay' vines had longer shoot lengths than the uninoculated 'Chardonnay' vines when fertilized with P ($P = 0.015$) (Supplemental Table S4, Fig. 2B, triangles). No difference in shoot length was seen at this timepoint in the non-P fertilized treatment ($P = 0.993$) (Supplemental Table S4, Fig. 2B, circles). The only treatment effect detected for the final shoot length of 'Chardonnay' was a result of P fertilizer; 'Chardonnay' grown with P fertilizer had longer final shoot lengths than 'Chardonnay' grown without P fertilizer ($P = 0.013$) (Supplemental Table S6).

Shoot biomass. Interactions of AM fungi × P fertilizer were detected for the shoot biomass of 'Merlot' ($P = 0.003$) (Supplemental Table S5), but not 'Chardonnay' ($P = 0.331$) (Supplemental Table S6), after 5 months of growth. Without added P fertilizer, inoculated 'Merlot' vines had greater shoot biomass than uninoculated 'Merlot' vines ($P = 0.043$) (Supplemental Table S3, Fig. 3A). No difference in shoot biomass was seen between inoculation treatments in the presence of P fertilizer ($P = 0.341$) (Supplemental Table S3, Fig. 3A). For 'Chardonnay', inoculation with AM fungi had no effect; only the addition of P fertilizer influenced the shoot biomass ($P = 0.001$) (Supplemental Table S6). 'Chardonnay' vines grown in the presence of P fertilizer had greater shoot biomass than vines grown without P fertilizer (Fig. 3A).

Root biomass. Interactions of AM fungi × P fertilizer were detected for root biomass of 'Merlot' ($P = 0.041$) (Supplemental Table S5), but not 'Chardonnay' ($P = 0.512$) (Supplemental Table S6). When grown without P fertilizer, there was no difference in the root biomass of inoculated and uninoculated 'Merlot' vines ($P = 0.971$) (Supplemental Table S3, Fig. 3B); however, when grown with P fertilizer, 'Merlot' inoculated with AM fungi had greater root biomass than uninoculated 'Merlot' ($P = 0.008$) (Supplemental Table S3, Fig. 3B). Root biomass of 'Chardonnay' was unaffected by any treatment in this study (Supplemental Tables S4 and S6, Fig. 3B).

Root-to-shoot ratio. Because the shoots and roots of each cultivar responded differently to AM fungal inoculation relative to the addition of P fertilizer, we examined root-to-shoot ratios. Interactions of AM fungal inocula × P fertilizer were detected for the root-to-shoot ratio of 'Merlot' vines ($P < 0.001$) (Supplemental Table S5), but not of 'Chardonnay' vines ($P = 0.186$) (Supplemental Table S6). When fertilized with P, the root-to-shoot ratio of 'Merlot' was greater when vines were inoculated with AM fungi compared with the uninoculated vines ($P = 0.002$) (Supplemental Table S3). There was no effect of AM fungal inoculation on the root-to-shoot ratio of 'Merlot' without P fertilizer ($P = 0.274$) (Supplemental Table S3). There were no effects of any treatment on the root-to-shoot ratio of 'Chardonnay' (all $P > 0.05$) (Supplemental Tables S4 and S6).

Tissue nutrients

Tissue P. AM fungal inocula ($P = 0.025$) and P fertilizer ($P < 0.001$) both affected the P found in the vine tissue of 'Merlot'; no interactions were detected ($P = 0.748$) (Supplemental Table S5). 'Merlot' vines inoculated with AM fungi had higher tissue P than uninoculated 'Merlot' (Table 1, Supplemental Table S5). As expected, 'Merlot' vines grown with P fertilizer had higher tissue P than vines grown without P fertilizer (Table 1, Supplemental Table S5). For 'Chardonnay', only P fertilizer ($P < 0.001$) (Table S6) had an effect; 'Chardonnay' vines grown with P fertilizer had higher tissue P than vines grown without P fertilizer (Table 1).

Tissue N. The addition of P fertilizer affected vine tissue N ($P = 0.018$) (Table S5) of 'Merlot'; 'Merlot' vines grown with P fertilizer had lower tissue N than 'Merlot' vines grown without P fertilizer (Table 1). The AM fungi inoculation did not influence 'Merlot' tissue N ($P = 0.579$) (Supplemental Table S5), and neither treatment influenced tissue N in 'Chardonnay' (all $P > 0.05$) (Supplemental Table S6, Table 1).

Tissue K. For both cultivars, vines grown with P fertilizer had higher tissue K (Table 1,

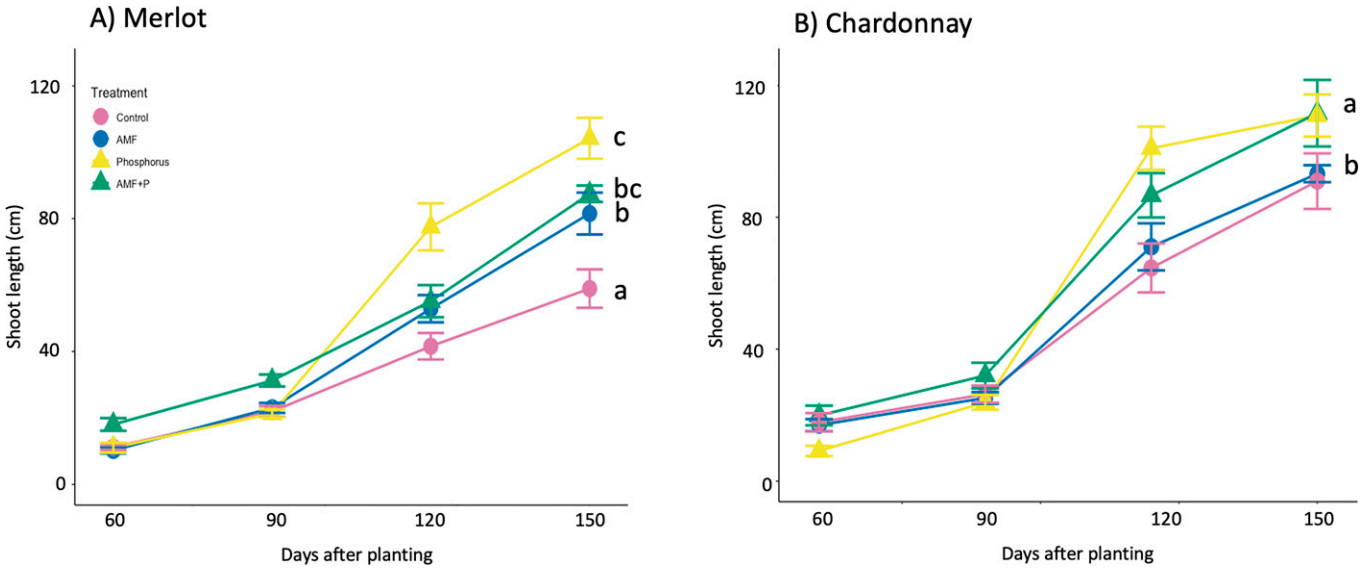

Fig. 2. Shoot length (cm) of *Vitis vinifera* (**A**) 'Merlot' and (**B**) 'Chardonnay' grown in a greenhouse for 5 months in the following treatments: (1) no AM fungi or phosphorus (P) fertilizer added (control; pink circles); (2) AM fungal inoculant added (AMF; blue circles); (3) P fertilizer added (P; yellow triangles); and (4) both AM fungal inoculant and P fertilizer added (AMF+P; green triangles). The triangles indicate vines that received P fertilizer. The circles indicate vines that did not receive P fertilizer. Shoot length was measured starting at 60 d after planting and every 30 d thereafter. Data shown represent means (±*SE*) of four observations until destructive harvest 150 d after planting. AMF × P fertilizer interactions were detected for shoot length at each time point measured for 'Merlot' (Supplemental Table S5), but only at 60 d for 'Chardonnay' (Supplemental Table S6). Letters indicate Tukey differences between means at the $P < 0.05$ level. Supplemental Fig. S2 shows the mean final shoot length (cm) for 'Chardonnay' (gold bars) and 'Merlot' (purple bars) at harvest (150 d after planting). Data points for individual plants are shown by dots within each column. Cultivar × AMF × P interactions ($P = 0.036$) are shown in Supplemental Table S2.

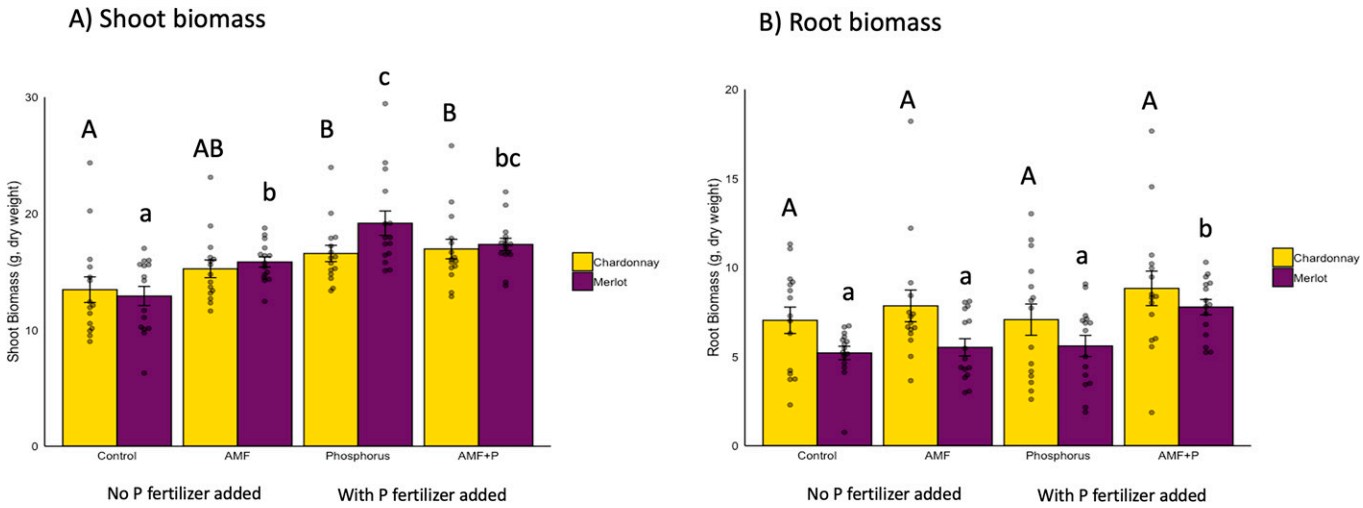

Fig. 3. Variations in (**A**) shoot biomass and (**B**) root biomass of young potted 'Chardonnay' (gold) and 'Merlot' (purple) grapevines to an arbuscular mycorrhizal (AM) fungal inoculant product (MycoBloom; MycoBloom LLC) without or with the addition of phosphorous (P) fertilizer. Plants were grown in 4-L pots in a greenhouse for 5 months. Columns show mean values ± *SE*. Dots within each column represent the biomass values for individual plants. Letters above the bars indicate Tukey differences between means at the $P < 0.05$ level. Uppercase letters show treatment differences within 'Chardonnay'. Lowercase letters show treatment differences within 'Merlot'.

Supplemental Tables S5 and S6). There was no effect of the AM fungal inoculation or the co-amendment on tissue K for either cultivar (Table 1).

Tissue Ca. There was an interaction of AM fungal inocula × P fertilizer treatment on tissue Ca for 'Merlot' vines ($P < 0.001$) (Supplemental Table S5). Vines grown with AM fungi and P fertilizer had higher tissue Ca than inoculated vines grown without P fertilizer (Table 1). Neither AM fungi nor the addition of P fertilizer influenced Ca uptake of 'Chardonnay' vines (all $P > 0.05$) (Supplemental Table S6, Table 1).

Mycorrhizal colonization of roots. The addition of P fertilizer reduced mycorrhizal colonization in the roots of both 'Merlot' ($t_{1,\ 13.5} = -13.69$; $P < 0.001$) and 'Chardonnay' ($t_{1,13.2} = -11.68$; $P < 0.001$) (Supplemental Table S7). When grown without P fertilizer, 'Merlot' vines had an average of 85% (*SE* ±2%) AM fungal colonization in their roots, and 'Chardonnay' had an average of 78% (*SE* ±4%) AM fungal colonization in their roots (Fig. 4). When P fertilizer was added, average mycorrhizal colonization levels decreased to 16% (*SE* ±5%) in 'Merlot' vines and 16% (*SE* ±4%) in 'Chardonnay' vines (Fig. 4).

Discussion

During a greenhouse study, we observed cultivar-specific responses to mycorrhizal inoculation in which newly rooted 'Merlot' grapevines had a stronger growth response to a mycorrhizal inoculant product (MycoBloom, LLC) than 'Chardonnay' vines, despite similar levels of mycorrhizal colonization in roots after 5 months of growth. In line with previous studies and model predictions (Johnson et al. 1997; Treseder and Allen 2002), when P fertilizer was added, mycorrhizal colonization levels of roots decreased significantly from an average of more than 75% root colonization in the absence of P fertilizer to an average of less than 20% root colonization in the presence of P fertilizer. We also observed an expected shift in the vine growth response to AM fungi inoculation in the different P fertilizer treatments. Inoculation with AM fungi increased aboveground biomass by 13% to 23% in vines that did not receive P fertilizer; however, when P fertilizer was added, inoculation with the AM fungal inoculant product had a neutral effect on vine growth. In contrast with our predictions, we observed a beneficial effect of the co-amendment of both AM fungi and P fertilizer on 'Merlot'; the co-amendment increased root biomass of 'Merlot' compared with the other treatments. This could mean that inoculating callused cuttings of 'Merlot' with an AM fungi product in a nursery setting combined with P fertilizer applications may be beneficial for young vine establishment and growth through the development of a larger root system. A larger root biomass in nursery stock at the time of planting may help to increase vine access to nutrients and water in the soil. This is supported by a previous study that showed that when 'Merlot' vines grafted onto the rootstock SO4 were inoculated with the AM fungal species *Glomus intraradices* (currently known as *Rhizophagus irregularis*) at the nursery stage and transplanted into the field, vines had significantly higher shoot biomass after 5 months compared with the uninoculated control vines (Nogales García et al. 2008). At 1 year later, differences in shoot biomass were still observed in the field between the preinoculated and uninoculated vines, and the plants that had been previously inoculated with *G. intraradices* at the nursery stage also showed higher chlorophyll levels in leaves (Nogales García et al. 2008).

Table 1. Average vine tissue nutrients (%) of the first-year, potted, own-rooted *Vitis vinifera* 'Merlot' and 'Chardonnay' grapevines 150 d after planting. Treatments include the following: control [without the addition of mycorrhizal inocula or phosphorus (P) fertilizer]; addition of an arbuscular mycorrhizal fungi (AMF) inoculant (MycoBloom; MycoBloom LLC); addition of P fertilizer (P); and a co-amendment of AMF+P. Data shown are means (*SE*).

		Tissue nutrients (%)			
Cultivar	Treatment	P	N	K	Ca
Merlot	Control	0.10 (0.01)	2.00 (0.12)	1.73 (0.04)	1.71 (0.09)
	AMF	0.18 (0.01)	1.98 (0.09)	1.72 (0.03)	1.38 (0.06)
	P	1.04 (0.04)	1.71 (0.09)	1.93 (0.07)	1.38 (0.08)
	AMF+P	1.15 (0.07)	1.80 (0.08)	1.89 (0.05)	1.61 (0.08)
Effects[i]	AMF	*	NS	NS	NS
	P fertilizer	***	*	***	NS
	AMF × P fertilizer	NS	NS	NS	***
Chardonnay	Control	0.09 (0.01)	1.68 (0.10)	1.70 (0.09)	1.39 (0.10)
	AMF	0.17 (0.01)	1.63 (0.09)	1.68 (0.05)	1.27 (0.07)
	P	0.86 (0.05)	1.57 (0.10)	2.08 (0.10)	1.39 (0.08)
	AMF+P	0.88 (0.05)	1.64 (0.09)	2.03 (0.08)	1.64 (0.20)
Effects[i]	AMF	NS	NS	NS	NS
	P fertilizer	***	NS	***	NS
	AMF × P fertilizer	NS	NS	NS	NS

[i] A type III analysis of variance (ANOVA) was used to determine significant main or interactive effects at $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and not significant (NS).

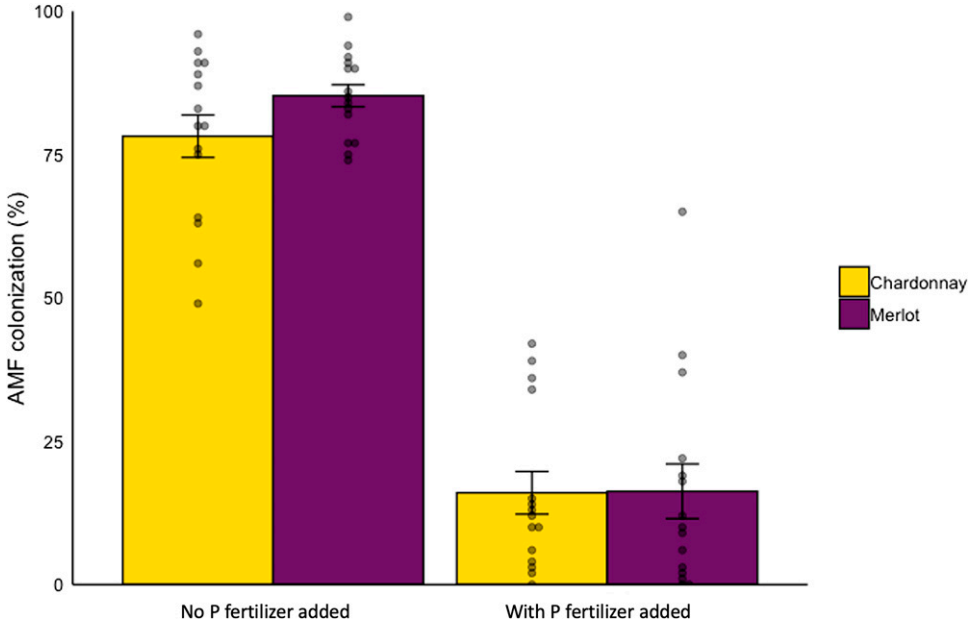

Fig. 4. Mean percentage arbuscular mycorrhizal fungal (AMF) colonization for 'Chardonnay' (gold) and 'Merlot' (purple) grapevines grown without the addition of phosphorus (P) fertilizer (left) and with the addition of P fertilizer (right). Plants were grown in 4-L pots in a greenhouse for 5 months. Columns show the mean and *SE*. Dots within each column show the percentage AMF colonization for individual plants. Uninoculated plants were confirmed to have no AMF colonization in roots via microscopy.

We found that inoculation with the AM fungal product provided small increases in tissue P in both cultivars. AM fungi inoculation increased tissue P from 0.10% to 0.18% in 'Merlot' and from 0.09% to 0.17% in 'Chardonnay' when the vines were not fertilized with P. Small increases in tissue P were also found for inoculated 'Merlot' vines with P fertilizer added, with tissue P increasing from an average of 1.04% in the P fertilizer treatment to an average of 1.15% in the treatment with both AM fungi and P fertilizer added. Although these results were not always statistically significant, inoculation of young grapevines in a nursery setting with an AM fungal inoculant product may be beneficial for improving the nutrient uptake of the vines before transplanting into the field. For example, more than 0.15% P is recommended for whole leaf tissue samples of wine grapes in Washington state (Moyer et al. 2018); in our study, inoculation with the AM fungal inoculant product increased tissue P to more than 0.15% in both cultivars.

Cultivar-specific grapevine responses to an AM fungal inoculant product. We found evidence of cultivar-specific responses of newly rooted grapevines to an AM fungal inoculant product. When no P fertilizer was added, 'Merlot' had greater aboveground biomass and longer shoot lengths when inoculated with the AM fungal product and when both the AM fungal inoculant product and P fertilizer were added as a co-amendment, 'Merlot' showed an increase in root biomass. However, the addition of the AM fungal inoculant product did not significantly improve or inhibit the growth of 'Chardonnay' under any P fertilizer condition. Our results for 'Merlot' are in concordance with those of Nogales García et al. (2008), who showed that nursery inoculation of 'Merlot' with the AM fungal species *G. intraradices* (currently known as *Rhizophagus irregularis*) resulted in greater shoot dry weight compared with uninoculated control plants for all five rootstocks tested (Richter 110, SO4, 41B, 14 Ru, and 1103 Paulsen).

Our results for 'Chardonnay', however, are in contrast with those of Linderman and Davis (2001), who found that inoculation with AM fungi increased the shoot growth of 'Chardonnay' 65% to 107% relative to uninoculated controls; however, we did not see this increase in shoot growth as a response to AM fungal inoculation (Fig. 2). Similar levels of 'Chardonnay' root colonization by AM fungi were also observed during the two studies (average 64% to 90% colonized in Linderman and Davis and average 78% colonized in the present study). A potential reason for the difference seen in the growth of 'Chardonnay' shoots between the two studies may be attributable to differences in the composition of the AM fungal inocula (Trouvelot et al. 2015). Although we used a mycorrhizal inoculant product that contained a mixture of seven AM fungal species, there was only one AM fungal species in our mixture, *Funneliformis mosseae* (formerly known as *Glomus mosseae*), that overlapped with the species of AM fungi used by Linderman and Davis (2001). The soil pH differences between the two studies also could have been a factor [pH 6.2 in Linderman and Davis (2001); pH 8.0 in this study] because soil pH is known to influence nutrient availability, but both soils had an acceptable pH range for own-rooted *Vitis vinifera* (Moyer et al. 2018).

Variations in growth response may be associated with differences in nutrient requirements between cultivars. Our research provides evidence that variations in growth responses to experimental treatments may be associated with differences in early-development P requirements between the 'Merlot' and 'Chardonnay' cultivars. This is best illustrated by the differences in shoot length and shoot biomass at the end of the experiment (Figs. 2 and 3A). At the end of the experiment, 'Merlot' vines grown in the P fertilizer treatment were much larger than 'Merlot' vines grown in the control treatment (Fig. 2A; yellow triangle vs. pink circle at 150 d on the line graph), demonstrating a strong growth response of 'Merlot' to the P fertilizer addition. However, there was only a small increase in the growth of 'Chardonnay' in the P fertilized treatment compared with the control (Fig. 2B; yellow triangle vs. pink circle on the line graph at 150 d), demonstrating that the addition of P fertilizer had less of an impact on 'Chardonnay' growth compared with 'Merlot'. Moreover, in the control treatment, 'Merlot' vines were substantially smaller than the 'Chardonnay' vines (Supplemental Fig. S2; gold bar vs. purple bar in the control treatment); however, when P fertilizer was added, the two cultivars grew to be similar in size (Supplemental Fig. S2; gold bar vs. purple bar in the P treatment). This provides further evidence that 'Merlot' may have been P-limited in the control treatment compared with 'Chardonnay'. Differences in responses to treatments were also observed for the final shoot biomass of 'Merlot' compared to 'Chardonnay' (Fig. 3A; gold vs. purple bars in the control and P treatments). 'Merlot' responded more positively to the AM fungal inoculant than 'Chardonnay' and had higher tissue P when inoculated with the AM fungal inoculant product compared with uninoculated vines. This suggests that 'Merlot' may have been able to meet its higher P requirements, either through the addition of P fertilizer or through symbiosis with AM fungi, which can increase root access to soil P.

This finding is in line with previous research that demonstrated that plants that benefit from nutrient additions can also benefit from inoculation with AM fungi (Cheeke et al. 2021) because associating with mycorrhizal fungi can increase the access to soil nutrients, especially P, by the plant.

Co-amendment of AM fungi and P fertilizer increased root biomass of 'Merlot'. The combination of P fertilizer and AM fungal inocula increased the root biomass of newly rooted 'Merlot' vines, but not 'Chardonnay'. During early vine growth and vineyard establishment, root growth is often preferred by growers to shoot growth because vines typically do not produce fruit until at least the second growing season (Keller 2015). Therefore, adding both AM fungal inocula and P fertilizer in a greenhouse setting may be beneficial for transplanting and may improve vineyard establishment for 'Merlot' vines. This potential growth benefit of adding both AM fungal inocula and P fertilizer has been observed during other studies. For example, tobacco yield was greater when AM fungi and NPK fertilizer were combined, compared with plants with either AM fungi or fertilizer treatment individually (Subhashini 2016). The combined treatment effect may be caused by nutrient mobilization and solubility by AM fungi, particularly of P and K, which tend to be immobile and, thus, unavailable to plants (Schachtman et al. 1998). Similar results were reported by Ziane et al. (2017) for tomato; inoculation by AM fungi and the addition of NPK fertilizer at a recommended rate of 50% together resulted in the same yield as that achieved with a recommended fertilizer rate of 100%. Because the grapevines in our study were younger than 1 year, we could not assess the effect of the AM fungal inoculant on fruit yield; however, at least one study found increased crop yield and quality of Crimson seedless grapevines with mycorrhizal inoculation (Nicolás et al. 2015). The results of our study and others indicate that AM fungal inoculants may be beneficial for supplementing fertilizer applications at the time of planting.

Our study showed that cultivar-specific responses to biotic and abiotic amendments. Our study also provided evidence that the co-amendment of an AM fungal inoculant in addition to P fertilizer may be beneficial for enhancing the root development of some cultivars, thus making it a potential advantage in the nursery setting before transplanting to the vineyard. Because our study identified cultivar-specific responses of young grapevines to a mycorrhizal inoculant product, it may be beneficial to test the efficacy of inoculant products on the cultivars of interest in a greenhouse setting before investing in biological inoculant products on a larger scale.

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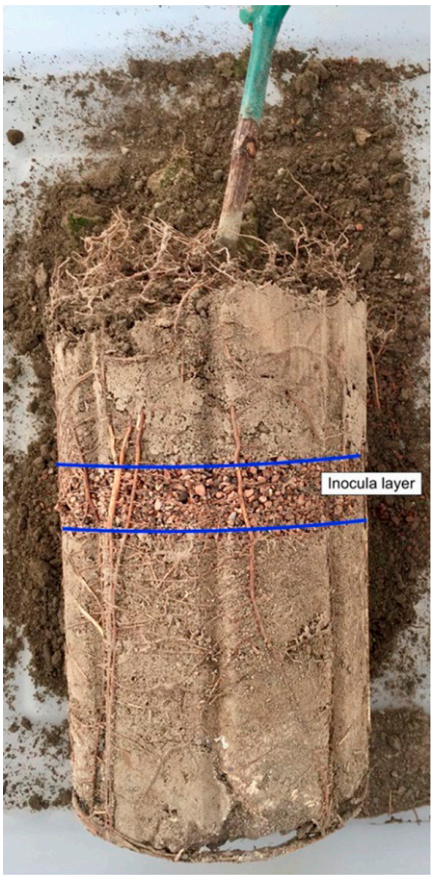
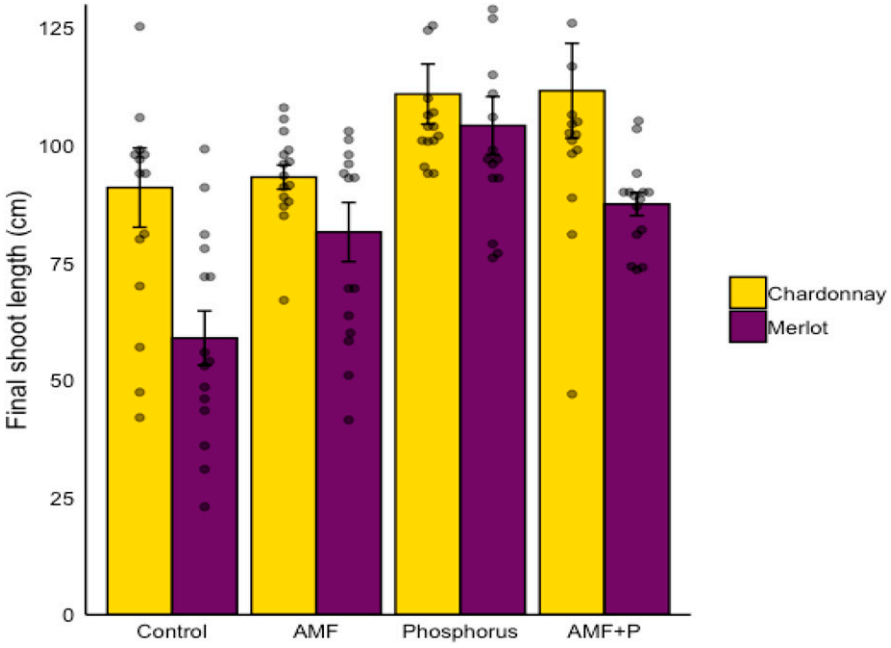


Supplemental Fig. S1. Experimental design for treatments receiving an inoculant containing arbuscular mycorrhizal (AM) fungi (MycoBloom; MycoBloom LLC, Lawrence, KS). Each inoculated pot (4 L) contained 400 mL of the AM fungal (AMF) inoculant product and 3600 mL of autoclaved 1:1 sand:soil mix (inoculated at a rate of 10% by volume). Treatments without the AMF-inoculated product received 400 mL of autoclaved sand:soil mix instead of the inoculant.



Supplemental Fig. S2. Mean final shoot length (cm) of *Vitis vinifera* cultivars Chardonnay (gold) and Merlot (purple) grown in the following treatments in a greenhouse for 5 months: (1) no arbuscular mycorrhizal (AM) fungi or phosphorus (P) fertilizer added (control); (2) AM fungal inoculant added (AMF); (3) P fertilizer added (P); and (4) after AM fungal inoculant and P fertilizer added (AMF+P). Shoot length was measured starting at 60 d after planting and every 30 d thereafter. Data shown represent means (±*SE*) of shoot length at harvest at 150 d after planting.

Supplemental Table S1. Soil characteristics of original agricultural background soil and final experimental soil mixed 1:1 by volume with sand and autoclaved.

Soils	pH	NO_3-N	NH_4-N	Olsen P	K	Ca	Mg	Fe	S
Background soil (n = 1)	7.9	4.0	1.5	31	214	8.2	3.3	7.0	3.0
Sand/soil mix (n = 5) [i]	8.0	2.6	7.1	10	271	11.4	1.1	4.2	6.2

All nutrients are expressed as $mg \cdot kg^{-1}$.

[i] Background agricultural soil was mixed 1:1 by volume with sand and autoclaved to kill existing soil microorganisms prior to being used in experimental treatments. Ca = calcium; Fe = iron; K = potassium; Mg = magnesium; N = nitrogen; NH_4 = ammonium; NO_3 = nitrate; P = phosphorus; S = sulfur.

Supplemental Table S2. Analysis of variance results for the full model with both *Vitis vinifera* cultivars, Merlot and Chardonnay showing the effects of cultivar, treatments [control, arbuscular mycorrhizal fungal inocula (AMF), phosphorus fertilizer (P), AMF+P)], and their interactions with the final shoot length, plant biomass, and tissue nutrients. The control treatment contained no AMF inocula or P fertilizer. The P fertilizer treatment contained no AMF inocula. The initial bud number at the time of planting was included as a covariate in the model to account for potential variations in cutting size before treatment. Plants were grown in their respective treatments in a greenhouse for 150 d (n = 15 replicates in each treatment). The sum of squares (Sum Sq), mean of squares (Mean Sq), numerator degrees of freedom (NumDF), denominator degrees of freedom (DenDF), F values, and *P* values are reported. Bold *P* values are significant.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	*P* value
Final shoot length (cm)						
Cultivar	6211.60	6211.60	1	111	10.08	**0.002**
AMF inoculation	267.60	267.60	1	111	0.43	0.511
Phosphorus fertilizer	15641.90	15641.90	1	111	25.39	**<0.001**
Initial bud number	2659.70	2659.70	1	111	4.32	**0.040**
Cultivar:AMF	9.70	9.70	1	111	0.02	0.900
Cultivar:phosphorus	482.30	482.30	1	111	0.78	0.378
AMF:phosphorus	3029.10	3029.10	1	111	4.92	**0.029**
Cultivar:AMF:phosphorus	2766.00	2766.00	1	111	4.49	**0.036**
Shoot biomass (g)						
Cultivar	18.45	18.45	1	99	2.29	0.133
AMF inoculation	20.74	20.74	1	97	2.57	0.112
Phosphorus fertilizer	298.16	298.16	1	97	36.99	**<0.001**
Initial bud number	1.25	1.25	1	107	0.15	0.695
Cultivar:AMF	2.24	2.24	1	97	0.28	0.599
Cultivar:phosphorus	17.15	17.15	1	97	2.13	0.148
AMF:phosphorus	70.71	70.71	1	97	8.77	**0.004**
Cultivar:AMF:phosphorus	21.14	21.14	1	97	2.62	0.109
Root biomass (g)						
Cultivar	89.03	89.03	1	98	15.86	**<0.001**
AMF inoculation	44.49	44.49	1	97	7.92	**0.006**
Phosphorus fertilizer	23.82	23.82	1	97	4.24	**0.042**
Initial bud number	6.22	6.22	1	105	1.11	0.295
Cultivar:AMF	0.00	0.00	1	97	0.00	0.983
Cultivar:phosphorus	4.01	4.01	1	97	0.71	0.400
AMF:phosphorus	14.38	14.38	1	97	2.56	0.113
Cultivar:AMF:phosphorus	1.70	1.70	1	97	0.30	0.584
Root-to-shoot ratio						
Cultivar	0.59	0.59	1	100	10.13	**0.002**
AMF inoculation	0.06	0.06	1	97	1.05	0.308
Phosphorus fertilizer	0.34	0.34	1	97	5.80	**0.018**
Initial bud number	0.36	0.36	1	111	6.12	**0.015**
Cultivar:AMF	0.01	0.01	1	97	0.26	0.614
Cultivar:phosphorus	0.01	0.01	1	97	0.12	0.734
AMF:phosphorus	0.63	0.63	1	97	10.79	**0.001**
Cultivar:AMF:phosphorus	0.05	0.05	1	97	0.91	0.341
Tissue P (%)						
Cultivar	0.36	0.36	1	100	16.51	**<0.001**
AMF inoculation	0.16	0.16	1	97	7.14	**0.009**
Phosphorus fertilizer	21.48	21.48	1	97	971.99	**<0.001**
Initial bud number	0.00	0.00	1	111	0.02	0.902
Cultivar:AMF	0.02	0.02	1	97	0.77	0.383
Cultivar:phosphorus	0.35	0.35	1	97	15.95	**<0.001**
AMF:phosphorus	0.00	0.00	1	97	0.11	0.738
Cultivar:AMF:phosphorus	0.01	0.01	1	97	0.66	0.417
Tissue N (%)						
Cultivar	1.27	1.27	1	111	8.82	**0.004**
AMF inoculation	0.01	0.01	1	111	0.07	0.787
Phosphorus fertilizer	0.64	0.64	1	111	4.49	**0.036**
Initial bud number	0.11	0.11	1	111	0.77	0.382
Cultivar:AMF	0.01	0.01	1	111	0.05	0.819
Cultivar:phosphorus	0.29	0.29	1	111	2.02	0.158
AMF:phosphorus	0.10	0.10	1	111	0.69	0.407
Cultivar:AMF:phosphorus	0.00	0.00	1	111	0.00	0.978
Tissue K (%)						
Cultivar	0.01	0.01	1	100	0.11	0.744
AMF inoculation	0.01	0.01	1	97	0.20	0.653
Phosphorus fertilizer	2.32	2.32	1	97	34.71	**<0.001**
Initial bud number	0.31	0.31	1	111	4.67	**0.033**
Cultivar:AMF	0.00	0.00	1	97	0.01	0.935
Cultivar:phosphorus	0.20	0.20	1	97	2.97	0.088
AMF:phosphorus	0.01	0.01	1	97	0.09	0.759
Cultivar:AMF:phosphorus	0.00	0.00	1	97	0.00	0.964
Tissue Ca (%)						
Cultivar	0.36	0.36	1	98	2.61	0.109
AMF inoculation	0.00	0.00	1	97	0.03	0.868
Phosphorus fertilizer	0.14	0.14	1	97	1.01	0.318
Initial bud number	0.06	0.06	1	108	0.40	0.527
Cultivar:AMF	0.10	0.10	1	97	0.73	0.395
Cultivar:phosphorus	0.38	0.38	1	97	2.72	0.102
AMF:phosphorus	1.62	1.62	1	97	11.77	**0.001**
Cultivar:AMF:phosphorus	0.07	0.07	1	97	0.54	0.465

Ca = calcium; K = potassium; N = nitrogen.

Supplemental Table S3. Estimated marginal (EM) means contrasts for *Vitis vinifera* cultivar Merlot. Contrast analysis of linear mixed-effects models with the presence of mycorrhizal inoculum and phosphorus (P) fertilizer as an interaction term and block as a random effect (n = 15 replicates in each treatment). Degrees of freedom (df), t ratio, and *P* values are reported for differences between the arbuscular mycorrhizal fungal inoculation treatment (AMF) and control and between the co-treatment of AMF and P fertilizer (AMF+P) and phosphorus fertilizer (P) treatment 150 d after planting. The uninoculated control treatment contained no AMF or no P fertilizer. The P fertilizer treatment contained no AMF inocula. Bold *P* values are significant.

Contrast	Estimate	*SE*	df	t ratio	*P* value	P fertilizer	Effect of AMF
Shoot length (60 d)							
AMF vs. control	−1.21	1.82	41	−0.66	0.910	No	0
AMF+P vs. P	6.74	1.82	41	3.70	**0.003**	Yes	**(+)**
Shoot length (90 d)							
AMF vs. control	0.89	2.17	41	0.41	0.976	No	0
AMF+P vs. P	9.72	2.17	41	4.49	**<0.001**	Yes	**(+)**
Shoot length (120 d)							
AMF vs. control	12.20	7.03	41	1.73	0.322	No	0
AMF+P vs. P	−21.50	7.03 4	1	−3.06	**0.020**	Yes	**(-)**
Shoot length (150 d)							
AMF vs. control	23.06	7.64	41	3.02	**0.022**	No	**(+)**
AMF+P vs. P	−16.25	7.64	41	−2.13	0.162	Yes	0
Shoot biomass							
AMF vs. control	2.94	1.07	41	2.74	**0.043**	No	**(+)**
AMF+P vs. P	−1.81	1.07	41	−1.69	0.341	Yes	0
Root biomass							
AMF vs. control	0.27	0.63	41	0.44	0.971	No	0
AMF+P vs. P	2.14	0.63	41	3.41	**0.008**	Yes	**(+)**
Root-to-shoot ratio							
AMF vs. control	−0.12	0.07	41	−1.83	0.274	No	0
AMF+P vs. P	0.26	0.07	41	3.94	**0.002**	Yes	**(+)**
Tissue P (%)							
AMF vs. control	0.08	0.06	41	1.42	0.495	No	0
AMF+P vs. P	0.11	0.06	41	1.88	0.254	Yes	0
Tissue N (%)							
AMF vs. control	−0.02	0.14	41	−0.15	0.999	No	0
AMF+P vs. P	0.09	0.14	41	0.64	0.920	Yes	0
Tissue K (%)							
AMF vs. control	0.00	0.06	41	0.02	1.000	No	0
AMF+P vs. P	−0.03	0.06	41	−0.51	0.956	Yes	0
Tissue Ca (%)							
AMF vs. control	−0.33	0.10	41	−3.38	**0.008**	No	**(-)**
AMF+P vs. P	0.23	0.10	41	2.35	0.103	Yes	0

Ca = calcium; K = potassium; N = nitrogen.

Supplemental Table S4. Estimated marginal (EM) means contrasts for *Vitis vinifera* cultivar Chardonnay. Contrast analysis of linear mixed-effects models with the presence of mycorrhizal inoculum and phosphorus fertilizer as an interaction term and block as a random effect (n = 15 replicates in each treatment). Degrees of freedom (df), t ratio, and *P* values are reported for differences between the arbuscular mycorrhizal fungal inoculation treatment (AMF) and control, and between the co-treatment of AMF and P fertilizer (AMF+P) and phosphorus fertilizer (P) treatment 150 d after planting. The uninoculated control treatment contained no AMF or no P fertilizer. The P fertilizer treatment contained no AMF inocula. Bold *P* values are significant.

Contrast	Estimate	*SE*	df	t ratio	*P* value	P fertilizer	Effect of AMF
Shoot length (60 d)							
AMF vs. control	−0.90	3.37	41	−0.27	0.993	No	0
AMF+P vs. P	10.67	3.38	41	3.16	**0.015**	Yes	**(+)**
Shoot length (90 d)							
AMF vs. control	−1.03	3.89	41	−0.27	0.993	No	0
AMF+P vs. P	8.32	3.90	41	2.13	0.160	Yes	0
Shoot length (120 d)							
AMF vs. control	7.08	9.10	41	0.78	0.864	No	0
AMF+P vs. P	−12.95	9.13	41	−1.42	0.496	Yes	0
Final shoot length (150 d)							
AMF vs. control	2.99	10.40	41	0.29	0.992	No	0
AMF+P vs. P	2.21	10.40	41	0.21	0.997	Yes	0
Shoot biomass							
AMF vs. control	1.83	0.98	41	1.86	0.261	No	0
AMF+P vs. P	0.46	0.99	41	0.47	0.966	Yes	0
Root biomass							
AMF vs. control	0.78	0.97	41	0.80	0.855	No	0
AMF+P vs. P	1.69	0.98	41	1.73	0.323	Yes	0
Root-to-shoot ratio							
AMF vs. control	−0.08	0.11	41	−0.74	0.881	No	0
AMF+P vs. P	0.13	0.11	41	1.15	0.660	Yes	0
Tissue P (%)							
AMF vs. control	0.08	0.05	41	1.61	0.383	No	0
AMF+P vs. P	0.02	0.05	41	0.42	0.974	Yes	0
Tissue N (%)							
AMF vs. control	−0.06	0.14	41	−0.41	0.976	No	0
AMF+P vs. P	0.06	0.14	41	0.46	0.967	Yes	0
Tissue K (%)							
AMF vs. control	−0.01	0.12	41	−0.12	0.999	No	0
AMF+P vs. P	−0.04	0.12	41	−0.34	0.987	Yes	0
Tissue Ca (%)							
AMF vs. control	−0.11	0.17	41	−0.63	0.921	No	0
AMF+P vs. P	0.26	0.17	41	1.50	0.444	Yes	0

Ca = calcium; K = potassium; N = nitrogen.

Supplemental Table S5. Analysis of variance results for *Vitis vinifera* cultivar Merlot showing the effects of the following treatments: control, arbuscular mycorrhizal fungi inocula (AMF), phosphorus fertilizer (P), and the co-treatment of AMF and P fertilizer (AMF+P) on the final shoot length (cm), shoot and root biomasses (g, dry weight), root-to-shoot ratio, and tissue nutrients (P, N, K, Ca) of Merlot. The control treatment contained no AMF inocula or P fertilizer. Initial bud number at the time of planting into treatments was a covariate in the model. Plants were grown in their respective treatments in a greenhouse for 150 d (n = 15 replicates in each treatment). Sum of squares (Sum Sq), mean of squares (Mean Sq), numerator degrees of freedom (NumDF), denominator degrees of freedom (DenDF), and *P* values are reported. Bold *P* values are significant.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	*P* value
Shoot length (60 d)						
Initial bud number	180.14	180.14	1	55	7.24	**0.009**
AMF	114.05	114.05	1	55	4.59	**0.037**
Phosphorus	159.81	159.81	1	55	6.43	**0.014**
AMF:phosphorus	237.21	237.21	1	55	9.54	**0.003**
Shoot length (90 d)						
Initial bud number	84.91	84.91	1	55	2.42	0.126
AMF	419.97	419.97	1	55	11.97	**0.001**
Phosphorus	176.55	176.55	1	55	5.03	**0.029**
AMF:phosphorus	292.60	292.60	1	55	8.34	**0.006**
Shoot length (120 d)						
Initial bud number	2005.00	2005.00	1	55	5.42	**0.024**
AMF	325.10	325.10	1	55	0.88	0.353
Phosphorus	6360.80	6360.80	1	55	17.20	**<0.001**
AMF:phosphorus	4245.40	4245.40	1	55	11.48	**0.001**
Final shoot length (150 d)						
Initial bud number	623.60	623.60	1	55	1.43	0.237
AMF	173.20	173.20	1	55	0.40	0.531
Phosphorus	10357.60	10357.60	1	55	23.74	**<0.001**
AMF:phosphorus	5793.80	5793.80	1	55	13.28	**0.001**
Shoot biomass						
Initial bud number	0.65	0.65	1	55	0.08	0.784
AMF	4.72	4.72	1	55	0.55	0.462
Phosphorus	225.26	225.26	1	55	26.24	**<0.001**
AMF:phosphorus	84.61	84.61	1	55	9.86	**0.003**
Root biomass						
Initial bud number	4.03	4.03	1	55	1.37	0.246
AMF	21.64	21.64	1	41	7.39	**0.010**
Phosphorus	22.57	22.57	1	42	7.70	**0.008**
AMF:phosphorus	12.98	12.98	1	41	4.43	**0.041**
Root-to-shoot ratio						
Initial bud number	0.12	0.12	1	55	3.78	0.057
AMF	0.07	0.07	1	42	2.21	0.145
Phosphorus	0.12	0.12	1	42	3.76	0.059
AMF:phosphorus	0.53	0.53	1	41	16.68	**<0.001**
Tissue P (%)						
Initial bud number	0.02	0.02	1	55	0.66	0.420
AMF	0.13	0.13	1	41	5.41	**0.025**
Phosphorus	13.23	13.23	1	42	545.93	**<0.001**
AMF:phosphorus	0.00	0.00	1	41	0.10	0.748
Tissue N (%)						
Initial bud number	0.06	0.06	1	55	0.39	0.536
AMF	0.02	0.02	1	55	0.12	0.734
Phosphorus	0.89	0.89	1	55	5.97	**0.018**
AMF:phosphorus	0.05	0.05	1	55	0.31	0.579
Tissue K (%)						
Initial bud number	0.21	0.21	1	55	7.28	**0.009**
AMF	0.00	0.00	1	42	0.12	0.731
Phosphorus	0.59	0.59	1	42	20.35	**<0.001**
AMF:phosphorus	0.00	0.00	1	41	0.14	0.707
Tissue Ca (%)						
Initial bud number	0.00	0.00	1	54	0.02	0.880
AMF	0.04	0.04	1	41	0.52	0.473
Phosphorus	0.04	0.04	1	41	0.55	0.464
AMF:phosphorus	1.20	1.20	1	41	16.47	**<0.001**

Ca = calcium; K = potassium; N = nitrogen.

Supplemental Table S6. Analysis of variance results for *Vitis vinifera* cultivar Chardonnay showing the effects of the following treatments: control, arbuscular mycorrhizal fungi inocula (AMF), phosphorus fertilizer (P), and the co-treatment of AMF and P fertilizer (AMF+P) on the final shoot length (cm), shoot and root biomasses (g, dry weight), root-to-shoot ratio, and tissue nutrients (P, N, K, Ca) of Chardonnay. The control treatment contained no AMF inocula or P fertilizer. Initial bud number at the time of planting into treatments was a covariate in the model. Plants were grown in their respective treatments in a greenhouse for 150 d (n = 15 replicates in each treatment). Sum of squares (Sum Sq), mean of squares (Mean Sq), numerator degrees of freedom (NumDF), denominator degrees of freedom (DenDF), and *P* values are reported. Bold *P* values are significant.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	*P* value
Shoot length (60 d)						
Initial bud number	5.40	5.40	1	55	0.06	0.802
AMF	354.53	354.53	1	55	4.18	**0.046**
Phosphorus	122.67	122.67	1	55	1.45	0.234
AMF:phosphorus	501.98	501.98	1	55	5.92	**0.018**
Shoot length (90 d)						
Initial bud number	38.38	38.38	1	55	0.34	0.563
AMF	197.47	197.47	1	55	1.74	0.192
Phosphorus	65.85	65.85	1	55	0.58	0.449
AMF:phosphorus	327.80	327.80	1	55	2.89	0.095
Shoot length (120 d)						
Initial bud number	1569.70	1569.70	1	54	2.53	0.117
AMF	127.70	127.70	1	41	0.21	0.652
Phosphorus	9851.80	9851.80	1	41	15.90	**<0.001**
AMF:phosphorus	1502.90	1502.90	1	41	2.43	0.127
Final shoot length (150 d)						
Initial bud number	2154.70	2154.70	1	55	2.68	0.108
AMF	100.40	100.40	1	55	0.12	0.725
Phosphorus	5286.50	5286.50	1	55	6.57	**0.013**
AMF:phosphorus	2.30	2.30	1	55	0.00	0.958
Shoot biomass						
Initial bud number	4.76	4.76	1	49	0.66	0.421
AMF	19.46	19.46	1	41	2.69	0.109
Phosphorus	85.26	85.26	1	41	11.79	**0.001**
AMF:phosphorus	7.00	7.00	1	41	0.97	0.331
Root biomass						
Initial bud number	3.37	3.37	1	49	0.48	0.493
AMF	22.47	22.47	1	41	3.18	0.082
Phosphorus	4.14	4.14	1	41	0.59	0.449
AMF:phosphorus	3.09	3.09	1	41	0.44	0.512
Root-to-shoot ratio						
Initial bud number	0.23	0.23	1	55	2.62	0.111
AMF	0.01	0.01	1	55	0.09	0.770
Phosphorus	0.22	0.22	1	55	2.51	0.119
AMF:phosphorus	0.16	0.16	1	55	1.80	0.186
Tissue P (%)						
Initial bud number	0.02	0.02	1	55	0.92	0.341
AMF	0.04	0.04	1	55	2.06	0.157
Phosphorus	8.15	8.15	1	55	419.10	**<0.001**
AMF:phosphorus	0.01	0.01	1	55	0.70	0.405
Tissue N (%)						
Initial bud number	0.05	0.05	1	55	0.38	0.539
AMF	0.00	0.00	1	55	0.00	0.971
Phosphorus	0.03	0.03	1	55	0.25	0.621
AMF:phosphorus	0.05	0.05	1	55	0.38	0.540
Tissue K (%)						
Initial bud number	0.12	0.12	1	55	1.19	0.281
AMF	0.01	0.01	1	41	0.10	0.748
Phosphorus	1.95	1.95	1	41	18.60	**<0.001**
AMF:phosphorus	0.00	0.00	1	41	0.02	0.877
Tissue Ca (%)						
Initial bud number	0.20	0.20	1	55	0.90	0.348
AMF	0.09	0.09	1	41	0.38	0.540
Phosphorus	0.48	0.48	1	41	2.13	0.152
AMF:phosphorus	0.51	0.51	1	41	2.29	0.138

Ca = calcium; K = potassium; N = nitrogen.

Supplemental Table S7. Analysis of variance results for mycorrhizal colonization of roots of *Vitis vinifera* cultivars Merlot and Chardonnay showing the effect of cultivar, phosphorus (P) fertilizer, and their interactions on the percentage mycorrhizal of roots at the end of the experiment. Initial bud number at the time of planting was included as a covariate in the model to account for potential variations in cutting size before treatment. Plants with no arbuscular mycorrhizal fungal (AMF) inocula added did not have any mycorrhizal colonization in roots and were removed from this analysis. Plants were grown in their respective treatments in a greenhouse for 150 d (n = 15 replicates in each treatment). Sum of squares (Sum Sq), mean of squares (Mean Sq), numerator degrees of freedom (NumDF), denominator degrees of freedom (DenDF), F values, and *P* values are reported. Bold *P* values are significant.

	Sum Sq	Mean Sq	NumDF	DenDF	F-value	*P* value
Both cultivars						
Initial bud number	65	65	1	54.92	0.32	0.573
Cultivar	112	112	1	43.53	0.56	0.460
Phosphorus fertilizer	64,509	64,509	1	41.40	318.97	**<0.001**
Cultivar:phosphorus	188	188	1	41.40	0.93	0.340
Merlot						
Initial bud number	381	381	1	27	1.99	0.170
Phosphorus fertilizer	35,984	35,984	1	27	188.17	**<0.001**
Chardonnay						
Initial bud number	37	37	1	27	0.17	0.680
Phosphorus fertilizer	29,016	29,016	1	27	136.50	**<0.001**